

The University of Chicago

A COMPARISON OF TISSUE REAC-
TIONS TO PULMONARY INFECTION
WITH TUBERCLE BACILLI IN ANI-
MALS OF VARYING RESISTANCE

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PATHOLOGY

By
ARTHUR J. VORWALD

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**A COMPARISON OF TISSUE REACTIONS TO PULMONARY
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A COMPARISON OF TISSUE REACTIONS TO PULMONARY INFECTION WITH TUBERCLE BACILLI IN ANIMALS OF VARYING RESISTANCE ¹

ARTHUR J. VORWALD

In tubercle-bacillus infection wide differences are seen in the native resistance of animals of various species, and an acquired resistance may modify an initial susceptibility. It is traditional that resistance and susceptibility to any disease are in part dependent upon the number and the types of cells that respond to the specific causative organism. This implies that individual variations in response to tubercle-bacillus infection are correlated with variation in cellular reaction in the tissues which harbor these bacilli.

To test this view, the native response of a variety of normal animals to equalized dosage of one and the same strain of tubercle bacilli at varying intervals after infection, was observed. Particular attention was paid to the following features: first, the localization and extent of the cellular reaction; second, the relation between the initial reaction and the subsequent course of the disease; and third, the character and number of cells taking part.

This study was confined to the reactions occurring in the lung. In spite of the relatively complex structure of this tissue, it is favorable for histological study of the response of the body to the tubercle bacillus, for several reasons. By intravenous injection of tubercle bacilli the traumatic lesions inseparable from all inoculations through direct puncture are avoided, and the lung acts as a filter for a very large part of the tubercle bacilli, so that primary tubercles are early formed and easily observed.

EXPERIMENTAL TECHNIQUE

Human-type tubercle bacilli (H37)² from a one-month-old culture were used. A weighed amount was triturated in a glass mortar until the mass

¹ From the Department of Pathology, University of Chicago, Chicago, Illinois.

² This was isolated at the Saranac Laboratory, Saranac Lake, in 1905 from the sputum of a patient with chronic ulcerative pulmonary tuberculosis, and it still possesses relatively high virulence for guinea pigs.

was reduced to a fine paste. After this, while grinding was continued, a drop of sterile normal saline solution was added from time to time until the paste was evenly dispersed. This suspension was then made up to the desired dilution (0.1 mgm. per 1 cc.), placed in a glass-stoppered bottle, and shaken gently to bring about a more even distribution of the bacilli. Samples were examined microscopically to ensure that the organisms were well distributed and that clumping was reduced to a minimum.

An intravenous injection of a standard dose (0.1 mgm. per kilogram body weight) of this suspension was made into a series of normal guinea pigs, monkeys, dogs, rabbits, rats, chickens and cats. At intervals of one day, one week and one month after injection, an animal of each species was killed, and the lungs fixed *in situ* by an intratracheal injection of Zenker's fluid. Serial sections, to be used for the study of the localization and extent of the cellular reaction, were cut in paraffin and stained with carbol-fuchsin for the tubercle bacilli. The nuclei were stained with dilute haematoxylin. For the cellular differentiation, tissues were imbedded in celloidin and sectioned. The tubercle bacilli and nuclei were stained in the same manner as in the paraffin sections. The counter-stains used were methyl-green in one series of slides, and eosin-azure in the other. In still another series, for contrast between the eosinophilic granules and the red-staining tubercle bacilli, orange G-azure was used.

THE LOCALIZATION OF THE CELLULAR REACTIONS

Krause, throughout all his papers on tuberculosis, has assigned a position of prime importance to the lymphoid tissue as a point of localization. He contends (1) that it is because of the unequal amount of this lymphoid tissue in the lungs of the rabbit and of the guinea pig that these two animals differ in the amount of pulmonary tuberculosis which they ultimately develop. The guinea pig develops much less lung tuberculosis because it is relatively poor in intrapulmonic lymphoid tissue, while the rabbit develops much more because it is rich in intrapulmonic lymphoid tissue. Krause has correlated this fact with the localization of tuberculous lesions in the lung of the child and of the adult, the childhood type being analogous to that of the guinea pig, and the adult type to that of the rabbit. Baldwin, Petroff and Gardner (2) are of the same opinion, that is, that the first tubercle, whatever the pathway of infection, develops within masses of lymphoid tissue. Rich (3) opposes this idea

in saying that "there is very good evidence that the normal lymphatic anatomy of the human lung at different age periods is of little importance in determining the distribution or character of tuberculous involvement or the degree to which the regional lymph nodes will be involved."

In the experiments here reported the early reactions in the guinea pig, monkey, rabbit, rat, chicken, and cat did not occur in strict relation to the above-mentioned structures. There was a beginning thickening of the alveolar walls by cells, which by their later increased accumulation and growth involved these structures. In the dog, on the other hand, the initial response occurred principally about the blood-vessel wall and in its progression narrowed the lumen of the vessel. This reaction may have taken place in the perivascular lymphatics, but there was little or no localization in the larger perivascular lymphatic aggregates. Krause (4) states that the localization in the lungs depends upon the number of the bacilli injected, and upon the concentration at any one place of quantities of bacilli that completely plug channels which carry particles out of the lung. In this experiment these factors seem to have been controlled. Embolization by bacterial masses was reduced to a minimum by adequate trituration of the bacilli in preparation of the suspension. Although the injection of 0.1 mgm. per kilogram body-weight would be a large local dose, such an amount when injected into the blood-stream, as in this experiment, is quickly dispersed and very small numbers become localized at one point.

It is not the purpose here to deny the fact that lymphatics play a rôle in primary tuberculous infection. There is sufficient data to support the view that foreign particles coming to the lung do enter the domain of the lymphatic channels and are concentrated in the major lymphatic agglomerates. Inert foreign particles, mainly in the form of suspensions of ink, and also suspensions of live coccoid bacteria have been injected into the animal body and their distribution throughout the tissues verified by numerous postmortem examinations. Lurie (5), in determining the fate of intravenously injected tubercle bacilli in the organs of rabbits, reports that the localization of the bacilli follows the distribution of particulate matter, but the relative position of the lung as a point of localization is still doubtful. With the tubercle bacillus, because of its morphology, the distribution may not be in strict accordance with that of small round carbon particles or coccoid bacteria. A mechanical factor, such as the relation between the tubercle bacillus and the calibre of the capillary lumen through which it is passing and the speed of progression through that lumen may possibly determine its localization.

Werigo (6), in studying the early reaction of the rabbit's tissues to the anthrax bacillus, recognized the importance of this mechanical factor where he says, "It seems very likely that this phagocytosis takes place by preference in the capillaries of the lungs where the bacteria, especially such large bacteria as the anthrax bacillus, can easily stop for some moments. Thanks to the mechanical causes."

Besides this mechanical factor a biological factor plays a rôle in the primary localization of tubercle bacilli. Just as with other organisms, so too with the tubercle bacillus there is an early interplay between the tissue constituents and the bacillus. This latter factor has been observed by Borrel (7), Weil (8) and by Vorwald (9) (10) in the earlier cellular reaction to tubercle bacilli injected intravenously into normal rabbits. The author (10) has shown that one hour after infection there was a definite polymorphonuclear leucocytic response with extensive phagocytosis of the bacilli by these cells. Subsequently the bacillus was usually carried by this phagocytosing cell into the alveolar-wall tissue, the lymphatics, or the alveolar space; in other cases the cell with its content remained localized in the capillary lumen.

The efficiency of the lungs as a filter for the injected tubercle bacilli was better realized by gross and microscopic observation of other tissues such as the liver, and kidneys of the respective animals. Examination of serial sections of these organs by Harms (11) revealed a decided decrease, when compared with the lungs of the same animals, in the number of stainable tubercle bacilli and in the extent of the cellular reactions to these bacilli.

The reason for the preponderance in the lung involvement in the animals of this series, particularly at the one-month period, may in part be due to the fact that that organ is better adapted for the multiplication of the bacilli. Corper, Lurie and Uyei (12) have suggested that, because of the higher oxygen tension of the alveolar air, the bacilli grow much more rapidly in the lung than in any other organ of the body. A series of experiments are being conducted in this department, at the present time, to determine the effect of massive atelectasis by bronchial stenosis in dogs on experimentally produced pulmonary tuberculosis in that animal. The results, thus far, indicate that the mechanism hypothesized by Corper and his associates is a factor of major importance in determining the progression of tuberculous lesions in the lung tissue.

There may be still other factors to explain the predominance of pulmonary tuberculosis, such as manner of introduction and nutritional requirements. In any event, the ability of the lungs to retain a major portion

of the tubercle bacilli injected intravenously is a factor of paramount importance in determining the early localization of the tubercle.

The lymphoid tissue, although not serving as a focus for early localization of the acid-fast bacilli, did show some change. It became increasingly more prominent in the one-week and the one-month periods, at which time it showed hyperplasia and numerous mitotic figures. Thus it seemed that tubercle bacilli, even though not present in the lymphoid foci, exerted a stimulating influence upon them bringing about a decided hyperplasia of their constituent elements. Maximow, commenting on this phenomenon in inflammation, expressed the opinion that it was such lymphoid foci that gave rise to the lymphocytes and thus, in part, to the polyblasts of lymphocytic origin appearing in the field of inflammation.

EXTENT OF CELLULAR REACTION

The volume of the cellular reaction in the lung tissue was determined by microscopic observation of serial sections. A number of representative reactions about tubercle bacilli, in each of the animals at the different periods, was followed through the sections from beginning to end, and in each case measured by means of a Filer screw-micrometer ocular. The tubercle in the majority of cases varied in shape, being often circular, ovoid, or extending through the tissues in a linear fashion. In order, therefore, to secure the average diameter of the cellular reaction it was necessary to take various measurements, first through its least and then through its greatest thickness. By averaging these measurements an approximation of the diameter of a corresponding circular reaction was obtained. By measuring thus the cellular reaction in each section through which the tubercle in question extended, and then by obtaining the total of these diameters and dividing it by the number of sections in which the reaction appeared, an average diameter for the whole tubercle resulted. In this manner the average diameter of from five to ten tubercles in each animal at each period was obtained. The results are recorded in table 1, and in chart 1 which is a graphic representation of the measurements obtained. The following facts should be noted in connection with it.

In the cat, at the one-day period, there were in the alveolar walls many isolated cells which had phagocytosed one or more tubercle bacilli. About these cells there was no reaction of the lung tissue and consequently no measurement could be obtained.

The dog at the same period responded with a slight perivascular accu-

mulation of cells not measurable in any way conforming with the rest of the series. However, to indicate that there was a reaction at the one-day period the cellular growth has been raised slightly above the zero point

TABLE 1

ANIMAL	AVERAGE SIZE AT ONE DAY	AVERAGE SIZE AT ONE WEEK	AVERAGE SIZE AT ONE MONTH
	<i>mm</i>	<i>mm</i>	<i>mm</i>
Guinea pig	105	325	diffuse growth
Monkey	141	223	746
Dog	*	109	548
Rabbit	105	119	434
Rat	74	163	160
Chicken	83	235	76
Cat	*	152	94

* Too limited to be measured.

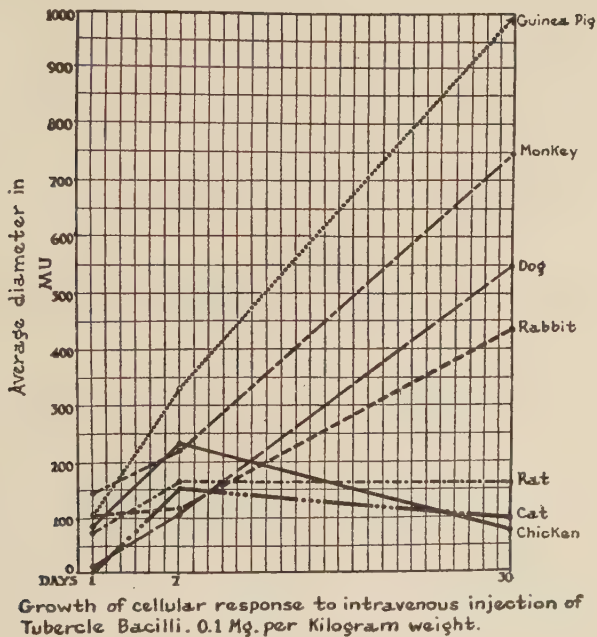


CHART 1

on the graph. At the one-week period the cellular reactions to the tubercle bacilli were still confined to the tissue immediately surrounding the blood-vessels, with no definite tubercle-formation. In order to measure

the extent of this cellular reaction it was necessary, first, to obtain the mean diameter of the blood-vessel and to subtract this from the mean diameter of the perivascular reaction including the blood-vessel. Thus the average diameter of the cellular reaction was obtained.

From observation of the chart, it appears that the reaction in the monkey at the end of one day surpasses that of all other animals at the same period. This was, however, the measurement of but one cellular response, the only reaction observed in the considerable number of sections examined. Many isolated cells were indeed found which had phagocytosed tubercle bacilli. These cells lay free in the lumen of blood capillaries and also in the alveolar walls. There was no reaction about them. The difficulty in evaluating the extent of response is obvious and for this reason the measurements of the single reaction were recorded. It thus does not represent an average of several diameters, as in the case of the other animals at this period.

The extent of the individual cellular reaction of the rat at the one-week and the one-month periods was recorded as practically the same. It might therefore appear that in this animal there was no progression of the disease. However, at one month there was a larger number of isolated cellular accumulations per given lung area than at one week, the ratio being as two to one. If the total volume of lung tissue involved were taken into consideration in the graph, progression at the later period would certainly be indicated.

The guinea pig at the end of one month responded not with isolated cellular accumulations but rather by a diffuse involvement of the whole lung tissue. The true curve representing the growth at this period should perhaps rise more sharply than indicated.

From the foregoing table and chart it is apparent that the extent of the response in the various species of animals is decidedly different. The ultimate outcome of the disease in the guinea pig and monkey on the one hand, and the rat and the chicken on the other, agrees quite well with the established ideas regarding the great susceptibility of the former group and the decided resistance of the latter two animals to human-type tubercle-bacillus infection. The final outcome of the disease in the rabbit also coincided with the general opinion that rabbits are less susceptible (more resistant) to human-type tubercle-bacillus infection than either the guinea pig or monkey. This inequality in reaction serves as a basis for routine differentiation of human- and bovine-type tubercle bacilli. With the other animals, however, such as the dog and the cat, study of experimental tubercle-bacillus infection has been less extensive than with

the previously mentioned species. There are scattered reports of spontaneous infection in dogs and cats. Fox (13) states that "tuberculosis occurs among domestic carnivores with reasonable frequency (3-5 per cent among dogs and 0.2-2 per cent among cats)." The opinion is prevalent that both the dog and the cat are relatively resistant to human-type tuberculosis. This study indicates, however, that our idea regarding the resistance of dogs to tubercle bacilli has been exaggerated. As recorded in the chart the dog's response at the one-month period was less than that of the very susceptible monkey but more than that of the moderately resistant rabbit.

The data presented are at some variance with other long and thorough investigations on the relation between the initial cellular reaction and the subsequent course of the disease. Krause (14), after pointing out that "there can be no virulence of germ without a corresponding susceptibility of animal," raises the question "are animals natively susceptible to virulent tubercle bacilli because their tissues are congenitally incapable of meeting first infection with promptness which is necessary to localize the germ?" and asks "Is it possible that the quality of virulence of germ is proportional inversely to the ability of tissue to react to it?" Krause (15) and Willis (16) explain the heightened resistance of the previously infected animal to a second infection on the ground that tubercle bacilli introduced into the body of an infected animal appear to be promptly localized by allergic reaction at the point of inoculation.

According to this view the outcome of the disease would presumably depend upon the extent of the initial cellular response, and one would expect those animals whose initial reaction is very meagre to develop the greatest amount of tuberculosis. This was, however, not the case. Such animals as the cat, the rat and the chicken whose early reactions were small, at the one-month period also showed the least tuberculosis. The guinea pig and monkey, on the other hand, reacted intensely within the first week, and a month later showed massive tuberculosis. The guinea-pig and the rabbit responses at the end of one day were very acute, and these animals should, therefore, according to the view cited, be relatively resistant. The guinea pig, however, proved the most susceptible animal of the group, while the rabbit was relatively more resistant than either the guinea pig, monkey or dog. The dog, whose early reaction was slightly in excess of that of the cat, developed much more tuberculosis ultimately.

This indicates that the individual variations in susceptibility of animals to tubercle bacilli are not dependent upon the initial cellular reaction.

One cannot, therefore, by observation of the early reaction of tissue cells to the bacilli, forcast the outcome of the disease.

CELLULAR DIFFERENTIATION

In the regions of inflammatory reaction certain types of cells, namely, polymorphonuclear leucocytes and typical lymphocytes and monocytes, were easy to identify. In the case of a larger number of cells, however, classification was not as simple. Mononuclear cells of varying appearance, with some characters in common with typical differentiated monocytes and with lymphocytes, but different in important respects from either, made up a large portion of the reacting mass in most tubercles. Cells of this group have been variously identified by observers as modified monocytes, clasmatocytes, histiocytes, polyblasts, etc. Much of the distinction has been based upon supravital staining, a method not applicable here, where the relation of the tubercle to previous anatomical structure was to be observed. It was, therefore, decided, for the time being, to include them all under the term mononuclear exudate cells, giving in each case an accurate description of the predominant type as they appeared in sections counterstained with eosin-azure. Differential counts for the whole series of animals follow.

Guinea pig

TABLE 2

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells.....	45.6	48.7	53.4
Mononuclear exudate cells with tubercle bacilli...	5.6	4.8	7.6
Typical differentiated monocytes.....	0.7	0.9	0.3
Lymphocytes.....	12.3	12.0	3.7
Plasma cells.....	0	0	0
Total polymorphonuclear neutrophilic* leucocytes.....	34.3	29.3	32.5
Polymorphonuclear neutrophilic* leucocytes with tubercle bacilli.....	5.5	7.1	17.3
True eosinophiles.....	0.9	0.7	0
Mitotic figures.....	0.6	0.4	0
Undetermined.....	5.1	5.7	4.3

* Pseudo-eosinophiles.

At one day the reactions consisted of well-localized thickenings of the alveolar septa by cells, with but slight exudation into the alveolar spaces. At one week the cellular accumulations had increased in size so that the septa were thickened for great distances and the air spaces were practically obliterated. By one month the reacting cellular mass had increased in size to such an extent that tubercles previously well isolated were beginning to coalesce. The alveolar spaces were now obliterated. There was no longer typical isolated tubercle-formation but rather a uniform involvement of the whole lung tissue, making measurements of individual reactions impossible. The majority of cells reacting throughout all periods were of the mononuclear-exudate type. Polymorphonuclear neutrophilic leucocytes (pseudo-eosinophiles), however, were prominent in all stages. Most of them lay in the alveolar spaces. There were numerous instances in which a cell of this type with its phagocytosed tubercle bacillus had been engulfed by a large mononuclear-exudate cell. The tubercle bacilli were everywhere within cells and in the later stages concentrated in the neutrophiles.

Monkey

TABLE 3

PERIOD	ONE DAY*	ONE WEEK	ONE MONTH
		<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells.		29.1	60.5
Mononuclear exudate cells with tubercle bacilli. . .		5.4	3.7
Typical differentiated monocytes.		0.2	0.2
Lymphocytes.		9.8	23.4
Plasma cells.		0	0.7
Total polymorphonuclear neutrophilic leucocytes. .		51.2	9.8
Polymorphonuclear neutrophilic leucocytes with tubercle bacilli.		8.9	0
True eosinophiles.		0	0
Mitotic figures.		1.3	0.4
Undetermined.		8.0	4.8

* See page 279.

At one day only one cellular reaction was observed and this in a paraffin section not stained for differentiation of cells. In a great many other

serial sections, made from stock at hand, no such reaction was observed. Instead, many isolated cells containing tubercle bacilli were found; however, there was no accompanying cellular reaction about them. Differentiation of these cells obtained from numerous eosin-azure stained sections is as follows:

- Mononuclear exudate cells in the alveolar walls containing tubercle bacilli, 13
- Monocytes in the blood-stream containing tubercle bacilli, 6
- Polymorphonuclear leucocytes containing tubercle bacilli, 8
- Mitotic figures in the blood-stream, 4

At one week cellular accumulations were easily observed. The alveolar septa showed isolated thickenings by cells and the neighboring alveolar spaces contained cells which lay free or grouped in small masses. The majority of cells making up the reaction at this period were polymorphonuclear neutrophilic leucocytes and the tubercle bacilli were confined mainly to these cells, but to a lesser degree were present in the reacting mononuclear-exudate cells. Just as in the guinea pig, so too in this animal many of the polymorphonuclear neutrophilic leucocytes with their phagocytosed tubercle bacilli were engulfed by mononuclear-exudate cells. Caseation at this one week period had not as yet taken place, but degeneration of cells was observed, especially of those collected in masses within the alveolar spaces. At one month there was typical tubercle-formation with central caseation. The caseating area contained much nuclear debris and great numbers of pycnotic mononuclear cells and degenerating polymorphonuclear leucocytes which could not be differentiated with certainty. The mononuclear exudate cells, now epithelioid, were larger than in the previous stages, and possessed round, eccentric nuclei poor in chromatin, and a granular eosinophilic and slightly foamy cytoplasm. The lymphocytes and plasma cells were concentrated about the periphery of the tubercle. The tubercle bacilli at this stage were concentrated in and about the caseating centre.

Dog
TABLE 4

PERIOD	ONE DAY*	ONE WEEK	ONE MONTH
		<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells.....		74.7	77.8
Mononuclear exudate cells with tubercle bacilli. . .		0.3	13.1
Typical differentiated monocytes.....		0.5	0

TABLE 4—*Concluded*

PERIOD	ONE DAY*	ONE WEEK	ONE MONTH
		<i>per cent</i>	<i>per cent</i>
Lymphocytes.....		7.4	9.9
Plasma cells.....		1.6	1.3
Total polymorphonuclear neutrophilic leucocytes.		11.2	5.4
Polymorphonuclear neutrophilic leucocytes with tubercle bacilli.....		0	0
True eosinophiles.....		0	0
Mitotic figures.....		0.9	4.7
Undetermined.....		3.4	0.6

* See page 281.

At one day the reactions consisted of slight perivascular accumulations of cells. In the many sections examined only two tubercle bacilli were observed. At one week there was a decided increase in the size of the individual perivascular accumulations. This increase was due mainly to mononuclear-exudate cells, about 50 per cent of which had a chromatin-rich nucleus with a slightly foamy basophilic cytoplasm. The remainder had larger and clearer nuclei with distinct nucleoli and pale blue foamy cytoplasm. At one month the reactions were concentrated about the much-compressed blood-vessels and the smaller respiratory bronchioles. There was no evidence of caseation. The tubercle bacilli in the earlier stages were absent or very difficult to find. At one month they were prominently distributed throughout the cells making up the cellular reaction.

Rabbit

TABLE 5

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells.....	72.0	74.2	63.1
Mononuclear exudate cells with tubercle bacilli...	2.2	4.4	0.4
Typical differentiated monocytes.....	0	0	0
Lymphocytes.....	2.2	9.3	17.2
Plasma cells.....	0	0.4	8.6

TABLE 5—*Concluded*

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total polymorphonuclear neutrophilic* leucocytes	14.3	10.4	5.0
Polymorphonuclear neutrophilic* leucocytes with tubercle bacilli	2.2	1.1	0.2
True eosinophiles	0	0.3	0
Mitotic figures	0.1	0.1	0.07
Undetermined	11.3	4.6	5.8

* Pseudo-eosinophiles or "specials."

At one day the interalveolar septa were in places markedly thickened, by cells mainly of the mononuclear-exudate type and to a lesser degree by polymorphonuclear leucocytes. At one week the picture did not deviate appreciably from that of one day except that there was more filling up of the air-spaces by cells of the mononuclear exudate type. At one month there was definite tubercle-formation with central caseation, enclosed by cells (mononuclear-exudate cells), whose nuclei were mainly ovoid and whose cytoplasm was eosinophilic and slightly foamy. These were typical epithelioid cells. About the periphery of the tubercle, lymphocytes and plasma cells were especially concentrated.

At the one-day period the tubercle bacilli were about equally phagocytosed by mononuclear-exudate cells and polymorphonuclear leucocytes (pseudo-eosinophiles). In many instances these pseudo-eosinophiles and their phagocytosed bacilli had been engulfed by mononuclear-exudate cells. Very often such phagocytosed cells showed dispersion of cytoplasmic granules and pycnosis of nuclei, leaving the tubercle bacilli lying in a clear space in the cytoplasm of the engulfing cell. At one month there was a decided increase in the number of observed bacilli per tubercle, and the bacilli were mainly concentrated in and immediately around the caseous centres.

Rat

TABLE 6

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells	74.6	78.5	78.8
Mononuclear exudate cells with tubercle bacilli	4.1	17.6	17.4

TABLE 6—*Concluded*

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Typical differentiated monocytes	9.5	2.4	4.1
Typical differentiated monocytes with tubercle bacilli	4.1	0	0
Lymphocytes	5.4	9.2	10.0
Plasma cells	0	0	0
Total polymorphonuclear neutrophilic leucocytes	8.1	5.2	1.6
Polymorphonuclear neutrophilic leucocytes with tubercle bacilli	1.3	0.4	0
True eosinophiles	0	0.1	0
Mitotic figures	0	1.2	1.6
Undetermined	2.05	2.9	2.7

At one day the cellular accumulations were confined principally to the alveolar walls, with little exudation into the alveolar spaces. The walls were thickened for the most part by mononuclear-exudate cells. At one week and one month there was more obliteration of the alveolar spaces by mononuclear-exudate cells which possessed centrally placed, round nuclei with distinct nucleoli. The cytoplasm was basophilic and not foamy. Others of these cells were larger and their cytoplasm was very foamy. At one month the tubercles, although approximately the same in size as those at the one-week period, were at this later stage more numerous. The tubercle bacilli were within mononuclear-exudate cells, mainly of the type with a basophilic nonfoamy cytoplasm. Others, however, were engulfed by the foamy type of cell, but decidedly in less number.

Chicken

TABLE 7

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells	77.7	78.5	87.0
Mononuclear exudate cells with tubercle bacilli	2.3	3.6	0
Typical differentiated monocytes	0.4	0.3	0
Lymphocytes	7.1	11.6	13.0
Plasma cells	0	0	0

TABLE 7—*Concluded*

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total polymorphonuclear neutrophilic* leucocytes	9.1	6.1	0
Polymorphonuclear neutrophilic* leucocytes with tubercle bacilli	0	0	0
True eosinophiles	0.2	0	0
Mitotic figures	0	0.1	0
Undetermined	5.2	3.1	0

* Spindle-shaped "crystalloid" eosinophiles.

The main type of cell throughout all periods was the mononuclear-exudate cell. At one day most of this type contained clear, slightly eccentric, irregular nuclei with a lining net-work which was attached to one, two or three distinct nucleoli. The cytoplasm stained pale blue and was not foamy. These cells were occasionally massed together, forming small but typical Langhans-type giant cells. At one week these mononuclear-exudate cells formed the centre of the tubercle. In general, they possessed more centrally placed, clearer nuclei and distinct nucleoli. The cytoplasm was faintly eosinophilic, and appeared granular and very slightly foamy. Lymphocytes penetrated this group of cells and surrounded the entire tubercle. At one month the tubercles were very much smaller and difficult to find. They consisted of a very compact group of mononuclear-exudate cells whose nuclei were similar to those at the one-week period. Their cytoplasm, however, was slightly granular and eosinophilic. At both the one-week and one-month periods there was degeneration but no frank caseation. The tubercle bacilli at one day were localized in groups of from ten to thirty in two or three mononuclear cells. At one week they were more evenly distributed but difficult to find. At one month they were absent.

Cat

TABLE 8

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total mononuclear exudate cells	48	53.8	86.7
Mononuclear exudate cells with tubercle bacilli	48	0.1	0.2

TABLE 8—*Concluded*

PERIOD	ONE DAY	ONE WEEK	ONE MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Typical differentiated monocytes with tubercle bacilli.	52	0	2.6
Lymphocytes.	0	4.5	3.7
Plasma cells.	0	0	0
Total polymorphonuclear neutrophilic leucocytes.	0	36.8	4.7
Polymorphonuclear neutrophilic leucocytes with tubercle bacilli.	0	0	0
True eosinophiles.	0	0.3	0
Mitotic figures.	0	0.1	0.4
Undetermined.	0	4.2	1.6

At one day there was no cellular reaction. Tubercle bacilli were found in occasional mononuclear cells, the majority of which were in the capillary lumen of the alveolar walls. These cells were differentiated as monocytes. The tubercle bacilli engulfed by such cells usually lay in a clear vacuole in no relation to the indentation of the nucleus. Others of these mononuclear cells lay in the alveolar walls and could not be differentiated as monocytes. They have been classified in table 8 as mononuclear-exudate cells. At one week and one month distinct cellular accumulations (small tubercles) were observed, which at the later period were smaller and fewer in number than at the one-week period. The tubercle bacilli at all stages were very difficult to find. At the later periods the small tubercles rarely contained a single bacillus and never more than one.

These data indicate that the cellular response in all animals at one day, one week and one month consisted mainly of mononuclear-exudate type cells. From observation of the nuclear and cytoplasmic structure and the relation between the two, it appeared that, in general, the mononuclear-exudate cells in the response at the earlier period differed somewhat from those partaking in the later reactions. At one day these cells in all animals varied in size and possessed eccentric, irregular, chromatin-rich nuclei and distinct nuclear walls. The cytoplasm stained a uniform pale blue with the eosin-azure technique and was not foamy. At one week the majority of mononuclear-exudate cells in the response of the guinea pig, rabbit, rat, and to a less extent the dog, were more uniform

in size, morphology and staining characteristics. In the chicken and the cat these cells possessed a larger, more ovoid, chromatin-poor nucleus with distinct nucleoli and nuclear walls. The cytoplasm was slightly foamy, with increased eosinophilia in those of the chicken. The exudate cells of the monkey, at this period, had a distinct foamy, slightly eosinophilic cytoplasm. This was also true for about half of those responding in the dog. At one month in the guinea pig, monkey, dog and rabbit the responding mononuclear cells had a large, more ovoid, chromatin-poor nucleus with distinct nucleoli. The cytoplasm was now foamy and moderately eosinophilic. Those of the cat were similar in type, except that they possessed a slightly more foamy cytoplasm. In the chicken the cytoplasm became more granular and eosinophilic. The rat responded with a type whose nuclei were chromatin-rich and slightly irregular and whose cytoplasm was both foamy and nonfoamy.

Sabin, Doan and Cunningham (17) in 1925 discriminated by supravital technique between two main types of mononuclear cells, the monocyte and the clasmatocyte. They advanced the hypothesis at that time that the monocytes retained the tubercle bacillus intact and afforded it opportunity to survive and multiply over long periods of time, while the clasmatocytes destroyed the bacillus. Sabin (18) and her coworkers consider the monocyte the source of the specific epithelioid cell of the tubercle.

Lewis (19), however, has shown by tissue culture that macrophages (clasmatocytes) become transformed into typical epithelioid cells. Hetherington and Pierce (20), also from tissue-culture experiments, have concluded that monocytes become transformed into clasmatocytes and that clasmatocytes in turn become epithelioid cells and Langhans-type giant cells. More recently Sabin (21) has stated that clasmatocytes and monocytes are very closely allied functional strains with many characteristics in common, and that it is possible for the monocyte to become a clasmatocyte. Gottlieb (22) has presented evidence to show that the tubercle bacillus is gradually digested in the cytoplasm of the monocyte. He looks upon the monocytic reaction in tuberculous infection as an attempted protective mechanism.

If Sabin, Doan and Cunningham's original hypothesis is correct one might expect that, in those animals, such as the guinea pig and monkey, which are very susceptible, the cellular reaction would be mainly of the monocytic type, while in the rat and chicken, animals more resistant, the clasmatocytic type of cell would be preponderant.

The supravital method was attempted in this experiment by a study of scrapings of the lung tissue. The inability, however, to obtain a preparation consisting of the cells of a tubercle, which in most animals, particularly at the early periods, was grossly not visible, made this method impracticable for a study of the cells making up the cellular reaction.

With fixed tissue stained by eosin-azure, the mononuclear-exudate cells making up the early stages of response in the various animals resembled somewhat the monocytes resting in the lumen of blood-vessels. In general, however, they were much larger than typical monocytes, their nuclei less rich in chromatin, and their cytoplasm less basophilic and more foamy. In the later stages there was no apparent similarity between the majority of mononuclear-exudate cells responding and typical monocytes. They possessed too many variations in nuclear and cytoplasmic structure and relationship. It was this latter fact that prompted Maximow to give the cells of this character the name *polyblasts*. He applied it because of the diversity of their appearance and their transformations. He believed that they arose mainly from transformed lymphocytes and monocytes of the blood-stream, and also in smaller part from the tissue cells *in situ*. If all these cells arose from fixed-tissue cells, then one might expect to find numerous cells undergoing mitotic division. In all observations of tissue of animals at the various stages the infrequency of mitotic figures in the field of inflammation was striking. This tends to support Maximow's contention that the majority of cells come from the blood-stream, unless extensive and rapid division by amitosis be admitted.

The polymorphonuclear neutrophilic leucocytes in the earlier stages were prominent in all animals except the dog and cat. In the remaining animals they played a major rôle in the phagocytosis of the tubercle bacilli, notably so in the guinea pig and the rabbit. These latter two animals responded in the beginning with large accumulations of polymorphonuclear leucocytes, which increased in the later stages in the guinea pig, but decreased in those of the rabbit. The striking persistence of these cells, in those animals which are most susceptible, namely, the guinea pig, the monkey and the rabbit, has some significance. Their rôle in tuberculosis has been particularly stressed by Medlar (23), and Medlar and Kastlin (24), who have emphasized their late appearance, with the onset of caseation, and correlated it with defeat of the host. In the experiment here reported the most susceptible animal, the guinea pig,

showed a persistent and slight increase in the number of polymorphonuclear leucocytes partaking in the reactions at the successive periods; true caseation, however, never resulted. In the monkey and the rabbit, caseation was present at the one-month period, but there was at this time a decided decrease in the number of polymorphonuclear leucocytes partaking in the individual responses, except perhaps in the caseating centre of the monkey's tubercle, where these cells could not be differentiated with certainty. In the chicken, on the other hand, a most resistant animal, degeneration and very slight caseation occurred at the one-week and one-month periods; there was throughout all stages a minimum polymorphonuclear leucocytic reaction. In the dog, rat, and cat caseation never occurred and the polymorphonuclear leucocytic response in all cases, except the cat at the one-week period, was always quite meagre. In brief this study indicates that the polymorphonuclear leucocytes appear early in the reaction, play a rôle in the localization of the bacilli, persist in the reaction of the most susceptible animals, and bear no definite relation to the process of caseation.

SUMMARY

Human-type tubercle bacilli in the proportion of 0.1 mgm. per kilogram body-weight were injected intravenously into guinea pigs, monkeys, dogs, rabbits, rats, cats and chickens. In all cases the lung tissue, in contrast to the intrapulmonic lymphoid tissue, retained a major portion of intravenously injected tubercle bacilli, and the initial cellular reaction occurred at the point of lodgment of the organisms. The lymphoid structures of the lung were involved only secondarily.

The susceptibility of the animals studied, as indicated by the measured extent of the individual cellular reactions, showed decided variations. The guinea pig and the rabbit, although in the beginning responding with an equally intense reaction, ultimately differed greatly as to the amount of tuberculous tissue developed. The guinea pig at one month proved to be a most susceptible animal, and the rabbit a much more resistant one. The rat and the chicken on the other hand, responding early with a reaction slightly less than that in the guinea pig and rabbit, at one month showed decidedly more resistance. The monkey (see pp. 279 and 280) and dog, which at one day showed a minimal response, less than that in any of the aforementioned animals, at the final period proved to have less extensive tuberculous reaction than the guinea pig, but much more

than the rabbit, rat, or chicken. The cat, the remaining animal of the series, reacted most sluggishly in the beginning, but, unlike the monkey and dog, in the final outcome proved a most resistant animal. The variations in susceptibility therefore did not parallel the intensity of the initial cellular reaction.

In the guinea pig, rabbit and rat the polymorphonuclear neutrophilic leucocyte played an important part in localizing the tubercle bacilli. Subsequently these leucocytes with their bacillary content were commonly phagocytosed by large mononuclear exudate cells. In the more mature reactions the mononuclear-exudate cell was the predominant type in all animals. The differentiation of these cells, in fixed tissues stained with eosin azure, into monocytes and clasmatocytes could not be made. No relation was detected between these mononuclear-exudate cells and the susceptibility to tuberculosis of the individual species studied.

There was an evident quantitative and also a qualitative difference in species reactivity, a quantitative one as shown by the difference in size of the cellular responses, a qualitative one in that in certain species polymorphonuclear leucocytes persisted in the responses throughout all periods. In other species these cells took very little part in the later reactions, and in the chicken, a resistant animal, they were absent in the response at the one month period.

No constant relationship was found between caseation and any particular type of cellular reaction.

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